This article contains Supplementary Material available at http://www.interscience.wiley.com/jpages/0002-9483/suppmat

Grant sponsor: University of Arizona.

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following Gilbert et al. (2004). For full sample details see Table 1 and Supplemental Table S.1. As an internal control for the results, at least two extractions were performed on different samples for each individual, each at least 6 months apart. The extracts were stored at −208C.

At the CPH laboratory, hair shafts (2–38 cm lengths, dependent on sample availability) were cut into * 2-cm pieces and submersed in 5% commercial bleach for 10 min. The bleach was removed with a pipette and the hair pieces were rinsed through consecutive washings with water, 99% ethanol, and water. The hair pieces were incubated in 200 µl 10 mM Tris/HCl, 140 mM NaCl, 3 mM $CaCl_2$, 50 mM DTT, 1% SDS, 0.1 mg Proteinase K, pH 8.0 at 558C. The hair was usually dissolved after 20 min. Nail samples were thinly sliced into small samples (0.5–1.0 mg), then decontaminated, rinsed, and digested as earlier. The resulting 200 µl hair/nail extracts were purified using the silica based QIAamp DNA mini kit (Qiagen, Venlo, The Netherlands) and eluted with 2 × 100 µl deionized, autoclaved water. The purified DNA was finally concentrated by centrifugation on a 30-kDa filter (Microcon, Millipore) and recovered from the filter with 80 µl autoclaved, deionized, and filtered water. The extracts were stored at −208C in 20 µl aliquots.

PCR a

The near complete mtDNA HVR1 region was PCR amplified from all DNA positive extractions using a number of different primer pairs that produce amplicons of between 135–141 bp (CPH) and 136–394 (AZ) (Table 2). In CPH 12.5 µl PCR reactions were performed containing 2.5 µl DNA extract, 2.5 µl High-Fidelity PCR Buffer (Invitrogen), 2.5 mM $MgSO_4$, 0.1 mM of each dNTP, 0.8 µM of each primer, 0.5 U Platinum Taq High-Fidelity polymerase (Invitrogen). The polymerase was activated by heating at 948C for 7 min, followed by 40 cycles of PCR (for details refer to Table 2). In AZ 25 µl PCR reactions were performed containing 1 µl DNA extract, 2.5 µl High-Fidelity PCR Buffer (Invitrogen), 2.5 mM $MgSO_4$, 0.2 mM of each dNTP, 1 µM of each primer, 0.2 U Platinum Taq High-Fidelity polymerase (Invitrogen). The reaction parameters were similar to those in CPH. In both laboratories PCR and extraction blanks were run at a 1:1 ratio.

The majority of the amplified products were cloned using the Topo TA kit (Invitrogen) or the pGEM[1] Easy Vector system (Promega). A small number of amplified products from AZ were direct sequenced without cloning.

DNA e

Multiple clones were sequenced for each cloned PCR reaction (see Supplemental Tables S.2 and S.3) using conserved primers and the ABI Prism 310 DNA single capillary DNA analyzer and the BigDye[1] Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems), following the manufacturers instructions. DNA sequences were aligned with the revised Cambridge Reference Sequence (rCRS) (Andrews et al., 1999) and analyzed for postmortem damage induced miscoding lesions, and the presence of contaminant DNA sequences. If no evidence of contamination could be observed, the multiple PCR fragments from the individual extractions were assembled into consensus contigs.

Re a Te a Ta Te PCR a a e

The DNA content of the extracts was quantified by quantitative real time PCR (qPCR) using the ABI Prism 7000 Sequence Detection System and the TaqMan Universal PCR Magg.8(an74)91(real)-389.3(time)-383.9(PCT563321.278754

bert et al., 2004), and in this situation although the samples are older than those that have been previously used in DNA studies, they are still relatively young with regards to other human aDNA studies (&600 years), and have been preserved under conditions that are optimal for DNA survival (cold and dry), thus rendering it likely that mtDNA will survive in the hairs.

2. Although the samples have been handled in the past, hair and nail has been demonstrated to be resistant to contamination (Tahir and Watson, 1995; Wilson et al., 1995; Jehaes et al., 1998; Anderson et al., 1999; Gilbert et al., 2006b), even when degraded, and in contrast to bone and teeth (Gilbert et al., 2006a) is relatively easy to successfully decontaminate (Gilbert et al., 2006b).
3. While some of the internal replicated DNA extracts did not yield amplifiable DNA, the sequence from each mummy was always generated from several different DNA extracts, in several cases from multiple tissues, using multiple overlapping independent PCR products, by two independent groups (see Supplemental Tables S.1, S.2, and S.3). This makes it unlikely that laboratory specific contamination, postmortem damage, heteroplasmy, or sequencing artifacts may have caused erroneous results.
4. The DNA levels within the samples are relatively low (Table 1 and supplemental information) in contrast to what would be expected from DNA from modern contaminant sources. Furthermore, the cloned sequences offer evidence of miscoding lesions that are believed to arise through DNA damage (Pääbo, 1989).
5. The generated sequences are phylogenetically plausible (see next section), fall within expected haplogroups, have a degree of diversity among them, and have never been previously generated in either laboratory.
6. The work was undertaken in dedicated aDNA labs, under strictly controlled conditions. No modern human DNA was used as controls.

DNA e e e

Contemporary Inuit haplotypes from Greenland and North Canadian Kitikmeot populations, that have not been subject to admixture with Europeans, all exclusively into two haplotype clusters, A2 and D3 (Helgason et al., 2006). The &600-year-old mtDNA haplotypes in this study are no exception: all fall within Hg A2 (Helgason et al., 2006). However three distinct subgroups are represented in the data (Table 1). Four individuals (I/2, I/3, II/6, II/8) belong to the root form of A2a (16111c-t, 16192c-t, 16223c-t, 16290c-t, 16319g-a, 16362t-c) (Helgason et al., 2006), while three (I/1, I/4, II/7) belong to a common, derived form of A2a (in the following referred to as A2a-311) that includes a further transition at 16311t-c (Helgason et al., 2006). The last individual (I/5) belongs to the root group of the related haplogroup A2b, lacking the 16192 c-t, but with derived 16265a-g (Helgason et al., 2006). Intriguingly, this result is different to that published in the only previous study to examine mtDNA from these specimens (Nielsen et al., 1994), where the sequence to mummy I/5 was reported as containing the 16192 c-t transition (in addition to the 16111c-t transition), whereas the 16223c-t transition was not observed (the sequence was only generated between 16099 and 16255). However, for a number of reasons we argue that our results likely represent the true sequence. Firstly, the 16223c-t transition is part of the sequence motifs for haplogroups A and D (Richards et al., 2000), and contemporary Inuit populations belong exclusively to subgroups of A2 and D3, both containing the 16223c-t transition (Helgason et al., 2006). Secondly, our sequence was derived from multiple extractions and PCRs on the individual. Thirdly, the mummies are reported to likely be contaminated with modern DNA (cf. Nielsen et al., 1994). To deal with this we used hair and nail as DNA sources, which are much easier to decontaminate from exogenous sources of DNA (cf. Tahir and Watson, 1995; Wilson et al., 1995; Jehaes et al., 1998; Anderson et al., 1999; Gilbert et al., 2006b) than sources such as bone (cf. Richards et al., 1995; Handt et al., 1996; Hofreiter et al., 2000; Kolman and Tuross, 2000; Gilbert et al., 2005b, 2006a; Malmström et al., 2005; Sampietro et al., 2006). Lastly, we did not use any modern Inuit DNA in our study, and have never worked on modern Inuit DNA, unlike in the previous study (Nielsen et al., 1994) where modern Inuit DNA was used as a control. As such, we believ913.4dy,

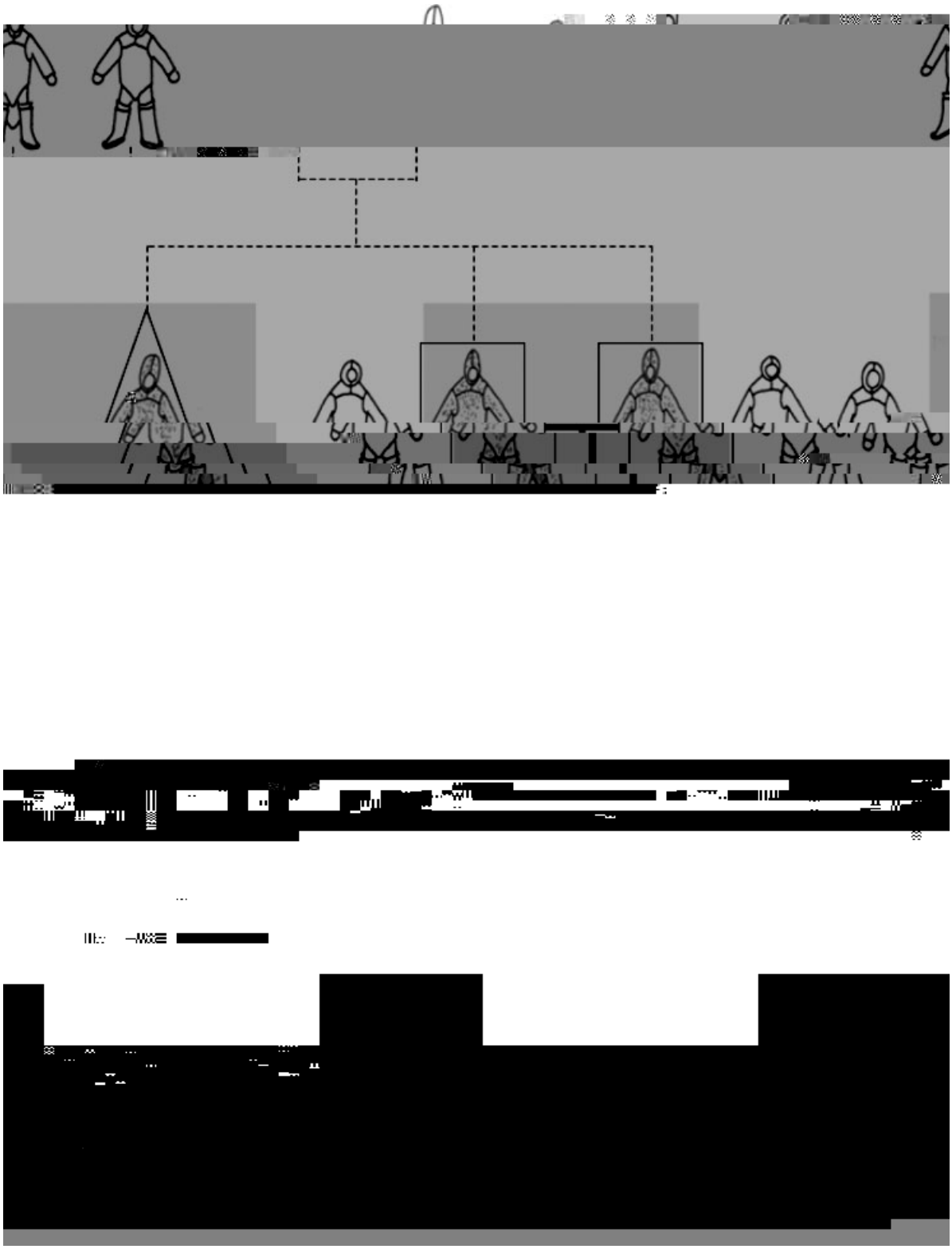
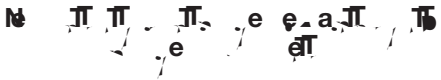


Fig. 1.

Although Helgason et al. (2006) noted that A2a is common to modern W. and S. Greenland indigenous populations, while A2b is predominantly found in Kitikmeot and N. Greenland, both forms are found widely across the region. Thus based on the small size of our data set, nothing can be concluded from the results other than that the samples are characteristically Inuit.

The age of origin of subhaplogroups A2a and A2b in Greenland has been calculated by Helgason et al. (2006) using a variety of colonization models (i.e. manipulation of founder vs. source population). The observation of A2a and A2b within our specimens fits within the calculated origin times. For example, under the model of a combined Kitikmeot and Siberian source population, Helgason et al. (2006) calculated the origin of A2a in Greenland as 426 ± 159 years (based on pedigree mutation rate) or 1,160 ± 434 (using an evolutionary rate model). Similarly they calculated the age of A2b 376 ± 160 or 1,024 ± 435. The presence, therefore, of both haplogroups A2a and A2b in the Qilakitsoq mummies that are nearly 550 years old clearly sets a lower boundary for the ages, thus the calculated dates of Helgason et al. (2006) can be modified accordingly.



Based on the mtDNA sequence data generated in this study we can conclude that, in contrast to the original hypothesis of two distinct family groups divided into two distinct grave piles (Hansen and Gürtler, 1983), the Qilakitsoq mummies represent at least three different genetic maternal lineages that are mixed between the piles (Table 1), and that we name according to the observed haplogroups: lineage A2b (mummy I/5), lineage A2a (mummies I/2, I/3, II/6, II/8), and lineage A2a-311 (mummies I/1, I/4, II/7). As similar mtDNA sequences may be shared between many individuals that are not directly related, it is not possible, using the mtDNA data alone, to further resolve whether the members of clusters A2a and A2a-311 represent direct maternal relatives. However, when the mtDNA data is compared with the previous HLA

-366.2(me)99uree9.5(-)rigiId(th)19vigeewhm4(b)023(e7.5a)0(tn30.9(t)0s(th)-232721(f)n.4(ay)-i1(i)14.-37164(D)0(N)12.4(b)0(e)1fo30(si)u